

Nuclease

Week 11

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

Summary

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| 1 | Inoculation of remaining BL21(DE3) liquid culture containing pSB1C3-T7-Nuc for IPTG induction | 3 |
| 2 | Kirby-Bauer test of expressed Nuclease | 4 |
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Definitions

BL21(DE3)

Top10

PCR

ddH₂O

pSB1C3-T7-Nuc

IPTG

TOB1

McConkey

SDS-PAGE

Kirby-Bauer

1 Inoculation of remaining BL21(DE3) liquid culture containing pSB1C3-T7-Nuc for IPTG induction

Responsible

Oscar He, Ellinor Lindholm and Oskar hman

Protocols used

Protein expression

Modifications and comments to protocols

	Nuc 2.1.2 GS BL21(DE3) 3	no IPTG
Protein expression (samples):	Nuc 2.1.2 GS BL21(DE3) 3	0.5 mM IPTG
	Nuc 2.1.2 GS BL21(DE3) 3	1.0 mM IPTG
Protein expression:	Incubation time	6 h
	Volume of TOB1 cells on McConkey plates	100 100 μ l

Experimental Setup

Table 1: Volumes of each liquid culture. From some, 1 ml was taken for optical density measurement at 660 nm.

Sample	Volume [ml]
Nuc 2.1.2 GS BL21(DE3) 3 - no IPTG	20
Nuc 2.1.2 GS BL21(DE3) 3 - 0.5 mM IPTG	20
Nuc 2.1.2 GS BL21(DE3) 3 - 1.0 mM IPTG	20

Results and Conclusions

No SDS-PAGE was done to test the presence and of Nuclease.

Discussion and Troubleshooting

The induced cell samples will be used for a Kirby-Bauer test.

2 Kirby-Bauer test of expressed Nuclease

Responsible

Oscar He, Ellinor Lindholm and Oskar hman

Protocols used

Kirby-Bauer

Modifications and comments to protocols

	Chloramphenicol	20 mg/ml
Protein/antibiotic samples:	Nuc 2.1.2 GS BL21(DE3) 3	no IPTG
	Nuc 2.1.2 GS BL21(DE3) 3	0.5 mM IPTG
	Nuc 2.1.2 GS BL21(DE3) 3	1.0 mM IPTG
Incubation time	16 h	
Volume of TOB1 cells on McConkey plates	100 μ l	

Experimental Setup

The samples were applied in duplicates on two different plates.

Results and Conclusions

The growth of the TOB1 cells were uneven and difficult to interpret.

Discussion and Troubleshooting

A new Kirby-Bauer test will be performed since the results from this one were not reliable.